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Urea synthesis in moderate experimental uremia

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Urea synthesis in moderate experimental uremia. Urea synthesis rates (USR) were examined in relation to individual variations in energy and nitrogen intakes. Rats made uremic by $^7/_8$ nephrectomy ($N = 12$) were pairfed with sham-operated controls ($N = 11$) and divided into two diet groups: diet 1 (4 kcal/g, 18% protein) and diet 2 (4 kcal/g, 42% protein). Nitrogen intake (NI) and energy intake (EI) were varied according to the quantity of feed given and the addition of a nonprotein gavage supplement. The USR was determined by ^{14}C-urea excretion during four periods when EI ranged from 20 to 50 kcal/day and NI ranged from 150 to 675 mg/day. Although USR did not correlate directly with either dietary protein or energy, the percent of protein-derived calories allowed the prediction of USR from NI. Fractional urea synthesis was not related to NI but rather to total EI. The nonlinear regression described a critical EI of 30 kcal/day below which USR increased to 75% of the NI. USR was not different between control and uremic animals. These data suggest an advantage in maintaining an appropriate protein:energy ratio (2.5 g per 100 kcal) to minimize the fractional urea synthesis. The utilization of nitrogen at different levels of protein and energy intake was not altered by the state of experimental uremia.

Synthèse de l'urée au cours de l'insuffisance rénale expérimentale. La synthèse de l'urée (USR) a été étudiée dans différentes situations d'apport énergétique et azoté chez des rats rendus urémiques par une néphrectomie des $^7/_8$ ($N = 12$). Ces rats ont été "pair-fed" à des rats témoins ($N = 11$). Les animaux ont été divisés en deux groupes, l'un recevant le régime 1 (4 kcal/g, 18% de protéine), l'autre recevant le régime 2 (4 kcal/g, 42% de protéine). L'apport azoté (AA) et énergétique (AE) a été modifié en variant les quantités de nourriture administrées et en ajoutant un supplément non protéique par gavage. USR a été déterminée par l'excrétion de ^{14} C-urée au cours de quatre périodes où AE allait de 20 à 50 kcal/jour, et AA de 150 à 675 mg/jour. Alors que USR n'est corrélée ni avec l'apport énergétique, ni avec l'apport protéique, le pourcentage de calories protéiques dans le total de l'apport énergétique permet de prédire Us à partir de AA. La synthèse fractionnelle de l'urée (USR/AA) est plus en rapport avec AE qu'avec AA. Dans la régression non linéaire entre USR et AE, il existe un seuil critique pour l'AE autour de 30 kcal/jour au dessous duquel USR/AA augmente rapidement pour atteindre 75% de AA. Aucune différence de USR/AA n'a été trouvée entre les rats urémiques et les rats témoins. Ces résultats suggèrent qu'il y a intérêt à maintenir un rapport protéine/énergie approprié (2,5 g/100 kcal) pour minimiser la synthèse de l'urée. L'urémie expérimentale ne modifie pas l'utilisation de l'azote aux différents niveaux d'apport protéique et calorique.

Urea is the major vehicle for the elimination of excess nitrogen from the body via the kidneys, with approximately 20% being subject to hydrolysis in the intestinal tract [1]. The kinetics of urea metabolism have been used recently as an indicator of nitrogen utilization in both uremic and normal man [2–5]. Some authors have estimated the protein catabolic rate from the urea appearance in patients with renal disease [4,5], although it requires further definition if it is to be used as a true reflection of dietary nitrogen utilization.

Absolute rates of urea synthesis in normal and uremic subjects have not been compared. Furthermore, relative roles of total energy intake and nitrogen intake as they affect rates of urea synthesis have not been well defined.

The present study was designed to determine the effects of both calorie and protein intake on urea synthesis, to define more clearly their interrelationships, and to determine any variance between the normal and experimental uremic state.

Methods

Young male Wistar rats, each weighing 200 to 220 g, were made uremic by $^7/_8$ nephrectomy with a two-stage operation modified from the technique described by Chantler, Lieberman, and Holliday [6]. Both stages were performed within 3 days using flank incisions. Control animals underwent sham operations, exteriorizing the kidneys. Data collection was begun 2 weeks following the initial surgery when all animals had recovered their preoperative weight. A 60% decrease in creatinine clearance was obtained in the nephrectomized animals.

Urea synthesis was measured according to the technique of McKinley et al [7]. To minimize gut urea hydrolysis, we treated all animals daily throughout the experimental period with 12.5 mg of neomycin administered by gavage. Preliminary studies confirmed that this dose of neomycin increased the urinary recovery of ^{14}C-urea from 64.9 ± 9.1 to 84 ± 12%.

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Urea synthesis was determined during the last 2 days of each 7-day test period. Animals were placed in metabolic cages, and urine samples were collected for two simultaneous 24-hour periods. The cages were rinsed several times with water to ensure complete urea collections. The urine samples were acidified with 1 N hydrochloric acid and preserved by freezing if assays were not performed immediately. Urinary ^{14}C excretion was determined on the first 24-hour collection in control animals and on the total 48-hour urine collection in uremic animals. The 24-hour urinary ^{14}C recovery in controls was 85.6 ± 16.4%, and the 48-hour urinary ^{14}C recovery in uremic animals was 82.5% ± 13%. There was no significant difference. Plasma samples were counted in both control and uremic animals at 24 and 48 hours, respectively, to confirm that essentially all ^{14}C had been excreted.

Blood samples for urea nitrogen were obtained at time zero when animals were placed in metabolic cages and at 24 hours. Simultaneous weights were also measured at time zero and 24 hours. Plasma and urinary urea concentrations were determined by the urease method [8] (Biochemica test combination, Boehringer, Mannheim Diagnostica).

^{14}C-urea (NEC-108), 2mCi/mmole, was obtained from New England Nuclear, Boston, Massachusetts. In all experiments, 5 μCi dissolved in physiologic saline were injected i.p. at time zero. The radioactivity injected was determined from the product of the volume injected and the activity of duplicate standards. The volume injected was determined by weighing the syringe before and after injection to an accuracy of 0.001 g.

The urea distribution volume V_d was measured by the distribution of injected tritiated water (1 mCi/g, New England Nuclear, Boston, Massachusetts) according to the technique of Fay et al [9]. In all experiments, 10 μCi, dissolved in physiologic saline, were injected i.p. after the animal had been on constant 18% protein diet for 7 days with free access to water. Blood samples were taken 3 to 4 hours following the injection and frozen if not assayed immediately. The radioactivity injected was determined as described above for ^{14}C urea. V_d was corrected for 95% plasma water in all instances.

Samples of plasma and urine to be assayed for ^{14}C and tritium were transferred quantitatively into 10 ml of Instagel (Packard, Downers Grove, Illinois), and its activity was counted in a standard three-channel Packard liquid scintillation counter. The activity was corrected for quenching by the channel ratio method and for counting efficiency by reference to count rates of ^{14}C- and ^{3}H toluene standards of known activity.

Calculations. Urea synthetic rate (USR) was calculated from equations based on the premise that, during a given time interval, urea synthesis is equal to the sum of urea excreted in the urine and broken down in the gut and the positive or negative accumulation of urea in body fluids. Gut urea hydrolysis may be minimized by the administration of antibiotics, but an immeasurable amount may escape through this route.

A correction for gut clearance (Gu) was obtained by taking the total injected ^{14}C-urea as the sum of Gu and urinary urea clearance after all ^{14}C had been cleared from the plasma. This assumes that $^{14}CO_2$ produced by hydrolysis of ^{14}C urea is not reincorporated into urea. This assumption has been verified by Rudman et al [10]. The correction factor (CF) for gut urea clearance could then be applied to the measured urinary urea excretion:

$$CF = \frac{Gu + Uu}{Uu} = \frac{\text{total } ^{14}C \text{ injected}}{\text{urinary } ^{14}C \text{ recovery}}$$

and

$$USR\ (0,t) = Uu \frac{^{14}C \text{ injected}}{^{14}C \text{ recovery}} + V_d\ (U_{p\ (t)} - U_{p\ (0)})$$

where USR (0,t) is the urea synthesis rate between time interval 0t; Uu, total urinary urea; V_d, urea distribution volume; U_p, plasma urea. USR was expressed as milligrams of urea nitrogen per 100 g of body wt per day. Fractional urea synthesis was then calculated as the fraction of urea nitrogen synthesized per gram of nitrogen intake.

Experiments. The rats were divided into two groups according to the diets they were to receive (Table 1). Both diets were similar in calorie density (3.8 kcal/g) but differed in their concentrations of protein, which was of high biologic value. Diet 1 contained 18% protein; Diet 2 contained 42% protein. A basic ration was provided to all rats, and only those who consumed their total ration throughout the 4-week period were included in the study. Calorie intake was further increased by the addition of an oral supplement of 10 kcal/day in the form of fat (Lipomul®) and carbohydrate (Maltrinex®) administered by gavage. The calorie distribution of this supplement was 90% fat and 10% carbohydrate.

Table 1. Experimental design

Period	Feed allotment *g*	Calories *kcal/day*	Group 1 (30 mg/g) nitrogen *mg/day*	Group 2 (67.5 mg/g) nitrogen *mg/day*
A	5	20	150	337
B	5 + supplement	30	150	337
C	10	40	300	675
D	10 + supplement	50	300	675

This allowed variation in the percentage of protein in the diet without varying the absolute quantity consumed.

The two diets consumed provided protein as herring fish flour, carbohydrate as corn starch, and saccharose and fat as peanut oil. Diet 1 contained 18% protein (nitrogen 30 mg/g of food), and diet 2 contained 42% protein (nitrogen, 67 mg/g of food) by weight. The distribution of calories for diet 1, was 19% protein, 19% fat, and 62% carbohydrate. For diet 2, it was 47% protein, 21% fat, and 32% carbohydrate. A complete mineral and salt mixture was provided.

The food was stored under refrigeration in a closed container and was weighed and distributed to each animal in cups provided with moveable lids to prevent spillage. Cages were inspected for spillage, which was negligible. No data were included if the animal failed to consume his alloted ration during the study period. Gavage was performed at the same time daily in all animals to administer equal volumes of calorie supplement or water.

Variations in calorie and protein intakes were established by studying each group of animals during the four periods of 7 days each. During periods A and C, both protein and total calories were varied by giving daily rations of 5 to 10 g, respectively, of their predesignated diet. During periods B and D, absolute protein intake was the same as in periods A and C, respectively, but total calories were varied by giving the previously described protein-free calorie supplement. All animals were studied in random sequence to avoid influencing the data by serial progression in nutrient intakes (see Table 1, Experimental design).

The study design allowed observations and controlled comparisons of urea synthesis during the suboptimal energy intakes habitual to chronically uremic subjects while normal dietary nitrogen requirements were maintained.

Statistical methods. Statistical differences between groups were determined by Student's *t* test [11]. Multiple regression analysis was performed as outlined by Downie and Heath [12].

Results

Experimental model. The 12 uremic and 11 control animals were assessed as to the validity of the experimental model by comparing weights, renal function, and isotope recovery (Table 2). Body weights remained stable or decreased slightly during the total 4-week study period. Weight gain was prevented by the limited energy intakes provided during periods A and B. Urea distribution volumes in uremic animals were slightly higher (0.68 ± 0.01) than they were in control animals (0.665 ± 0.02), but the difference was not significant.

Partial nephrectomy significantly decreased the creatinine clearances of experimental animals (uremic, 15.9 ± 1.7 ml/hr per 100 g of body wt; control, 40.3 ± 4 ml/hr per 100 g of body wt).

Total ^{14}C recovery for both groups was similar and comparable with values obtained by McKinley et al [7] in rabbits treated with neomycin. Gut urea clearance for control and uremic animals was also similar: control, 13.2 ± 16%; 17.6 uremic ± 12%.

Urea synthesis: Relative role of total energy and nitrogen intake. Multiple regression analysis allowed a numerical assessment of the relative effects of both total energy intake and nitrogen intake on urea synthesis (Table 3). The multiple correlation

Table 2. Comparison of uremic and control rats[a]

	Wt_i *g*	Wt_f *g*	ΔWt *g*	V_d	C_{Cr} *ml/hr/100 g body wt*	^{14}C recovery %	Gu
Control ($N=11$)	264 ± 8	237 ± 9	−27 ± −7.5	0.67 ± 0.02	40.3 ± 4.0[b]	87 ± 5	13 ± 16
Uremic ($N=11$)	234 ± 7	227 ± 9	−6.8 ± 7.0	0.68 ± 0.01	15.9 ± 1.7	83 ± 4	18 ± 12

[a] Values are the means ± SEM. Abbreviations are defined as: Wt_i, initial weight; Wt_f, final weight; Δ Wt, mean weight change; V_d, urea distribution as a fraction of body weight; C_{Cr}, creatinine clearance; Gu, gut clearance of urea expressed as percent of ^{14}C injected.
[b] $P < 0.01$

Table 3. Relative effects of nitrogen intake (x_1) and energy intake (x_2) on urea synthesis (y)[a]

Multiple regression analysis	Control (N = 8)	Uremic (N = 8)[b]	P
r_{x_1y}	0.64	0.69	NS
r_{x_2y}	−0.31	−0.28	NS
$r_{x_1x_2}$	0.47	0.47	NS
$r_{x_{12}y}$	0.98	0.94	<0.01
$r_{x_2y \cdot x_1}$	−0.90	−0.89	<0.01
$r_{x_1y \cdot x_2}$	0.94	0.97	<0.01

[a]The calculations for this table are as follows, where partial correlation coefficients (N = 8) are defined as y = urea synthesis = 1; x_1 = nitrogen intake = 3; and x_2 = calorie intake = 2:

$$r_{12\cdot3} = \frac{r_{12} - (r_{13}\, r_{23})}{\sqrt{(1-r_{13}^2)\,(1-r_{23}^2)}}$$

$$r_{12\cdot3} = \frac{-0.31 - (0.30)}{\sqrt{(0.59)(0.78)}} = \frac{-0.61}{0.68}$$

$$r_{12\cdot3} = -0.897 \text{ or } -0.90$$

[b]Mean values from each period were analyzed. Total observations were as follows: controls, 37; uremics, 42.

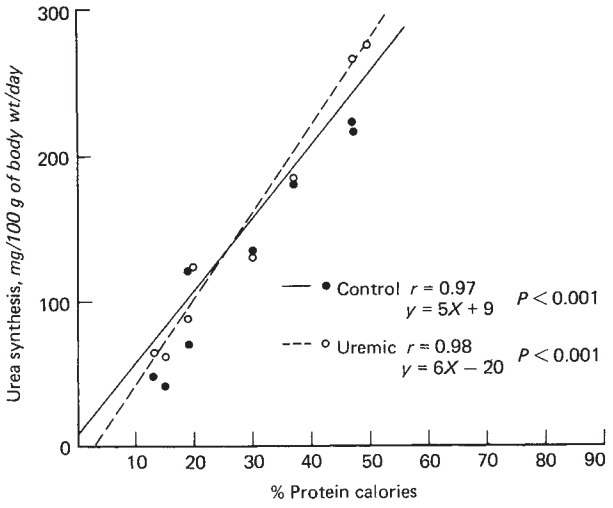

Fig. 1. *Variation of urea synthesis rate with protein-derived energy.*

coefficient (r) for these three variables was 0.98 in control animals and 0.94 in uremic animals.

Individual correlations of urea synthesis (y) with either nitrogen intake (x_1) or total energy intake (x_2) were not significant. But, when the partial correlation coefficient was used, with nitrogen intake held constant, calorie intake correlated significantly with urea synthesis: control, $r_{x_2y \cdot x_1} = -0.90$; Uremic, $r_{x_2y \cdot x_1} = -0.89$.

Likewise, when energy intake was held constant, the effect of nitrogen intake on urea synthesis was highly significant: control, $r_{x_1y \cdot x_2} = 0.94$; Uremic, $r_{x_1y \cdot x_2} = 0.97$. A positive significant correlation was demonstrated between urea synthesis and percent protein calories: control, r = 0.97; uremic, r = 0.98 (Fig. 1).

Urea synthesis at all levels of energy and nitrogen intake was similar in uremic and control animals (Table 4).

Fractional urea synthesis: Relative effects of total energy and nitrogen intakes. Fractional urea synthesis was expressed as the fraction of ingested nitrogen metabolized to urea daily (milligrams of urea nitrogen per milligram of nitrogen intake). There was no significant correlation between nitrogen intake and fractional urea synthesis: control, r = −0.35; uremic, r = −0.38.

A reciprocal nonlinear relation was observed between the fractional urea synthesis and the incre-

Table 4. Data summary of urea synthesis and fractional urea synthesis with variations in total energy (kcal/day) and nitrogen intake[a]

	Diet 1				Diet 2			
	A	B	C	D	A	B	C	D
Diet data								
Caloric value, *kcal/day*	20	30	40	50	20	30	40	50
Nitrogen intake, *mg/day*	150	150	300	300	337	337	675	675
N (control)	5	5	4	5	4	5	4	5
N (uremic)	5	5	6	6	5	5	6	4
Urea synthesis, *mg/100 g body wt/day*								
Control	121 ± 45	48 ± 11	73 ± 10	52 ± 20	218 ± 68	135 ± 18	223 ± 48	180 ± 51
Uremic	122 ± 21	64 ± 22	88 ± 35	62 ± 21	275 ± 105	130 ± 56	266 ± 76	184 ± 17
Fractional urea synthesis, *mg/g of nitrogen intake/day*								
Control	805 ± 299	323 ± 70	243 ± 35	173 ± 66	646 ± 200	402 ± 143	330 ± 59	286 ± 88
Uremic	811 ± 140	429 ± 147	292 ± 117	207 ± 70	815 ± 311	385 ± 167	395 ± 112	273 ± 25

[a]Values are the means ± SD.

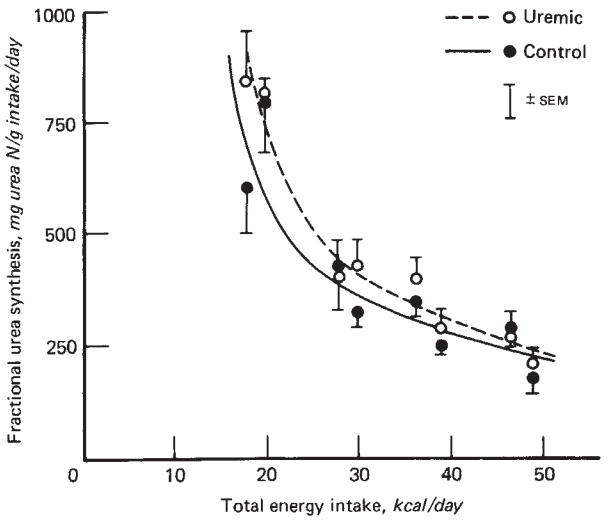

Fig. 2. *Nonlinear regression of fractional urea synthesis with energy intake.*

ments in total energy intake (Fig. 2). The break in the relation occurred at the point on the curve when total energy intake was 30 kcal/day. Energy intakes below this level resulted in a marked rise in fractional urea synthesis, with greater than 80% of the ingested nitrogen being diverted to urea. But, energy intakes of greater than 30 kcal/day appeared to suppress urea synthesis to less than 25% of the ingested nitrogen.

The semilogarithmic regression of the above relation was highly significant ($P < 0.001$) for both control and uremic animals: uremic, log Fs = -0.02 EI + 3.13, $r = 0.95$; control, log Fs = -0.02 EI + 3.18, $r = 0.94$, where Fs is fractional synthesis rate.

Discussion

The observations of this study offer the first experimental comparison of urea synthesis between chronically uremic and pair-fed control animals. Studies of patients with uremia have repeatedly demonstrated an anorexia resulting in suboptimal nitrogen and energy intakes associated with poor growth and negative nitrogen balance [13–16]. These experiments were conducted during periods of limited energy intakes that were inadequate to maintain normal growth. The study design allowed, however, observations and controlled comparisons of urea synthesis during the suboptimal energy intakes habitual to chronically uremic subjects while normal dietary nitrogen requirements were maintained. Holliday et al [17] showed in moderately uremic rats that carbohydrate feeding alone was able to abolish an exaggerated catabolic response to short-term fasting. The current study supports this finding, because an increase in nonprotein calories resulted in a decrease in urea synthesis. Similarly, there was no discernible difference between uremic or control animals.

Fractional urea synthesis derived by urea kinetics can offer an important index of nitrogen metabolism in uremic subjects. The fractional urea synthesis can be considered an indirect measure of protein catabolic rate because it reflects the quantity of urea produced for a given nitrogen intake. The nonlinear relation of energy and fractional urea synthesis (Fig. 2) demonstrates that the urea synthesis rate (USR) is not only directly dependent on dietary nitrogen but inversely dependent on total energy intake. This fact has frequently been stressed by Munro [18] in protein balance studies. Moreover, the relation defines a critical energy intake that will minimize USR and perhaps "spare nitrogen" for anabolic purposes. When dietary energy fell below 30 kcal/day, urea synthesis increased to more than 80% of ingested nitrogen, suggesting an increase in protein catabolism. When energy intake was maintained above 30 kcal/day, USR seemed to stabilize at approximately 25% of the nitrogen intake.

A similar relationship was observed in a clinical study of chronically uremic children during supplemental intravenous nutrition [19].

The direct relation of USR to protein-derived calories (Fig. 1) may offer a simple and readily available measure for predicting USR in the clinical setting if future human studies are substantiative. Traditionally, nutritional studies in renal failure have measured responses to different quantities of protein intake without note of the relative energy intake [20, 21]. Varcoe et al [22] recently reported a higher efficiency of urea nitrogen utilization in uremic subjects on a low (30 g) protein diet as compared with a normal (70 g) protein diet. But, there was no mention of total energy intake or the protein:energy ratio, which may have been the unmeasured variable [23].

The relationship of USR to protein-derived calories is also provocative in the dilemma of whether low-protein or high-protein diets are beneficial to the uremic animal. Low-protein intake improves survival [24, 25] but may limit growth and nitrogen balance [15, 16]. One wonders if the protein:energy ratio is the more important factor. If adequate energy is supplied quantitatively, more protein could be provided while maintaining a constant

protein:energy ratio and, therefore, a constant rate of urea synthesis.

Summary. These data have examined the importance of energy intake on urea synthesis in uremic and control animals. Urea synthesis is most dependent on the protein:energy ratio, which may prove to be a useful predictive measure in the clinical setting. Fractional rates of urea synthesis increase markedly with the stress of inadequate calorie intake, suggesting a catabolic response at a critical level of energy intake. No differences between moderately uremic and control animals were demonstrated in terms of urea synthesis. No differences in urea synthesis were apparent between moderately uremic and control animals during periods of similar dietary intakes.

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References

1. WALSER N, BODENLOS L: Urea metabolism in man. *J Clin Invest* 38:1617, 1959
2. ABITBOL C, HOLLIDAY M: Total parenteral nutrition in anuric children. *Clin Nephrol* 5:153–158, 1976
3. WASSNER SJ, ORLOFF S, HOLLIDAY MA: Protein catabolism in normal and uremic rats (abst). *Pediatr Res* 10:445, 1976
4. GOTCH FA, SARGENT JM, LAM M, PROWITT M, GRADY M: Clinical results of intermittent dialysis therapy (IDT) guided by ongoing kinetic analysis of urea metabolism. *Trans Am Soc Artif Organs* 22:175, 1976
5. SARGENT J, GOTCH F, BARAH M, PIERCY L, SPINOZZI N, SCHOENFELD P, HUMPHREYS M: Urea kinetics. A guide to nutritional management of renal failure. *Am J Clin Nutr* 31:1696–1702, 1978
6. CHANTLER C, LIEBERMAN E, HOLLIDAY M: A rat model for the study of growth failure in uremia. *Prediatr Res* 8:109–113, 1978
7. MCKINLEY JE, GILBERT DB, CHAO PY, REEVE ED: Urea metabolism and distribution in rabbits treated with neomycin. *Am J Physiol* 218:491–497, 1970
8. FAWCETT JK, SCOTT JE: A rapid and precise method for the determination of urea. *J Clin Pathol* 13:156–9, 1960
9. FAY JM, SCHNEIDER H: Estimation of total body water (virtual tritium space) in the rat, cat, rabbit, guinea pig, and man and the biological half life of tritium in man. *J Physiol* 154:169, 1960
10. RUDMAN D, DIFULDO TJ, GALAMBOS JT, SMITH RB, SALAM AA, WARREN DW: Maximal rates of excretion and synthesis of urea in normal and cirrhotic subjects. *J Clin Invest* 52:2241–2249, 1973
11. SNEDECOR G, COCHRAN W: *Statistical Methods* (6th ed.). Iowa State University Press, Ames, Iowa, 1972, p. 59
12. DOWNIE N, HEALTH R: *Basic Statistical Methods* (3rd ed.). New York, Harper and Row, p. 119–121
13. SIMMONS JM, WILSON CJ, POTTER DE, HOLLIDAY MA: Relation of calorie deficiency to growth failure in children on hemodialysis and the growth response to calorie supplementation. *N Engl J Med* 285:653–656, 1971
14. BETTS PR, MAGRATH G, WHITE RHR: Role of dietary energy supplementation in growth of children with chronic renal insufficiency. *Br Med J* 1:416–8, 1977
15. KOPPLE JD: Dietary requirements, chapter 15 *Clinical Aspects of Uremia and Dialysis,* edited by MASSEY SG, SELLERS AL, Illinois, C. C. Thomas, 1976, pp. 453–489
16. KOPPLE JD, COBURN JW: Metabolic studies of low protein diets in uremia: I. Nitrogen and potassium. *Medicine* 52:583–595, 1973
17. HOLLIDAY MA, CHANTLER C, MACDONNELL R, KIETJE J: Effect of uremia on nutritionally induced variations in protein metabolism. *Kidney Int* 11:236–245, 1977
18. MUNRO H: Energy and protein intakes as determinants of nitrogen balance. *Kidney Int* 14:313–315, 1978
19. ABITBOL CL, HOLLIDAY MA: Effect of energy and nitrogen intake upon urea production in children with uremia and undernutrition. *Clin Nephrol* 10:9–15, 1978
20. SCHOLZ A, KLUTHE E, KESSEL M: Investigations on the rate of urea synthesis and on nitrogen balance in patients with advanced chronic renal failure. *Proc EDTA* 7:161, 1970
21. COTTINI E, GALLINA D, DOMINGUEZ J: Urea excretion in adult humans with varying degrees of kidney function fed on milk, egg or amino acid mixture assessment of nitrogen balance. *J Nutr* 103:11, 1973
22. VARCOE AR, HOLLIDAY D, CARSON ER, RICHARDS P, TAVILL AS: Anabolic role of urea in renal failure. *Am J Clin Nutr* 31:1601–1607, 1978
23. HOLLIDAY M, CHANTLER C: Metabolic and nutritional factors in children with renal insufficiency. *Kidney Int* 14:306–312, 1978
24. GIORDANO C: Use of exogenous and endogenous urea for protein synthesis in normal and uremic subjects. *J Lab Clin Med* 62:231, 1963
25. GIORDANO C, ESPOSITO C, DE PASCALE C, DESANTO N: Dietary treatment in renal failure. *Proc 3rd Int Congr Nephrol* 3:214, 1967